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Review

Relationship between gut microbiota and development of T cell associated disease

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ABSTRACT

The interplay between the immune response and the gut microbiota is complex. Although it is well-established that the gut microbiota is essential for the proper development of the immune system, recent evidence indicates that the cells of the immune system also influence the composition of the gut microbiota. This interaction can have important consequences for the development of inflammatory diseases, including autoimmune diseases and allergy, and the specific mechanisms by which the gut commensals drive the development of different types of immune responses are beginning to be understood. Furthermore, sex hormones are now thought to play a novel role in this complex relationship, and collaborate with both the gut microbiota and immune system to influence the development of autoimmune disease. In this review, we will focus on recent studies that have transformed our understanding of the importance of the gut microbiota in inflammatory responses.

1. Regulation of the immune response by the gut microbiota

The commensal bacteria, or microbiota, colonizing the gut performs several functions: it plays a critical role in the breakdown of indigestible complex plant polysaccharides, synthesizes important nutrients such as vitamin K, and provides an important layer of defense against invasion by pathogenic microorganisms. Interestingly, the intestinal microbiota affects the immune and/or inflammatory status of the host by modulating intestinal barrier function and by influencing the development of the immune response. Several gut microbial structures that play an important role in barrier functions have been identified. The secreted protein, p40, from Lactobacilli LGG ameliorates cytokine-mediated apoptosis and disruption of the gut epithelial barrier [1], and flagellin from *Escherichia coli* Nissle is associated with induction of β-defensin 2 in epithelial cells [2]. Gut microbiota has been shown to direct maturation of the host immune system [3], to play a key role in the induction of immunoglobulin (Ig) A [4,5] and germinal centers [6], and to drive Th1, Th17, and regulatory T cell (Treg) development in the gut [7–9]. In most individuals, the commensal-mediated induction of these different components of the immune response is beneficial for host health. However, the composition of the gut microbiota can differentially influence various immune cell populations and adversely affect autoimmune/inflammatory disease-susceptible hosts, e.g., the presence of segmented filamentous bacteria (SFB) has been associated with a strong Th17 response and development of Th17-mediated diseases [10–12].

Colonic commensal bacteria may "educate" both thymically and peripherally-derived regulatory T cells. One study using mice transgenic for T cells expressing a limited, but diverse [i.e., express identical T cell receptor (TCR) β], TCR repertoire showed that the TCR repertoire of colonic Tregs is unique (i.e., is not expressed by thymically-derived Tregs) and reacts to bacterial isolates, suggesting that encounter with gut commensal bacteria induces peripheral generation of colonic Tregs, and thereby, tolerance to the gut microbiota [13]. In contrast, a more recent study also using transgenic mice expressing a limited, but diverse, TCR repertoire (i.e., TCR^{mini} mice) showed that thymic and intestinal Tregs share a majority of dominant TCRs and many of these TCRs recognize microbial antigens. Moreover, treatment of the TCR^{mini} mice with a cocktail of antibiotics that alters the composition of the gut microbiota results in a concomitant change in the colonic thymic Treg TCR repertoire. These results suggest that the repertoire of thymus-derived Tregs is heavily influenced by the microbiota, and thymus-derived Tregs may play a dominant role in maintaining tolerance to gut commensal bacteria antigens [14]. The contradictory results of these two studies make it difficult to reach any

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definitive conclusions about the pre-dominant source of the Treg populations, i.e., thymic-derived versus peripherally-induced, that reside in the gut and mediate tolerance to components of the gut microbiota. Other populations of regulatory cells also appear to be activated/induced by commensal bacteria. A population of B regulatory cells (Bregs) was identified in B6 mice whose gut bacterial load was reduced by oral administration of a combination of broad spectrum antibiotics [15]. Importantly, this Breg population protected the animals from developing experimental autoimmune encephalomyelitis (EAE). EAE protection by reduction of the gut microbiota is associated with generally enhanced levels of $CD19^+B220^+$ cells in the mesenteric/cervical lymph nodes (LN) and spleen, increased percentages of a subpopulation of $CD19^+CD5^+$ B cells in the mesenteric/cervical LN, and higher frequencies of the $CD1d^{hi}CD5^+$ cell subset in the spleen/LN. Adoptive transfer of splenic and LN $CD5^+$ B cells isolated from antibiotic-treated mice conferred better protection against EAE than $CD5^+$ B cells from untreated mice by inducing a shift from a Th1/Th17 to a Th2-response [15] possibly through a mechanism involving IL-10. Interestingly, reduction of the gut bacterial load by broad spectrum antibiotics protects against EAE in different models via induction of different populations of regulatory cells, e.g., IL-10-producing $CD1d^{hi}CD5^+$ Bregs are induced in B6 mice [15] and IL-10-producing Tregs in SJL mice [16]. It is not clear why a reduction in gut bacterial load would favor regulatory T and B cells versus effector T cells. However, the antibiotics may not only reduce the bacterial load, but they may also alter the microbial composition, thereby, favoring commensal bacteria that induce regulatory cells and/or eliminating commensal bacteria that induce Th1/Th17 cells.

In the past several years, numerous reports have shown that an alteration in gut microbiota can favor induction of effector T cells over Tregs, and, consequently, trigger the development of autoimmune/inflammatory diseases (reviewed in [17]). Those studies identified specific gut commensals that appear to induce either Th17 or Treg responses that are associated with development or protection from disease, respectively. The presence of segmented filamentous bacteria (SFB) in the murine gut is associated with induction of Th17-mediated autoimmune/inflammatory diseases, such as colitis (induced by transfer of $CD4^+CD45RB^{hi}$ cells into SCID mice), arthritis and experimental autoimmune encephalomyelitis (EAE) [10–12]. On the other hand, SFB protects from invasion by the pathogenic microorganism, *Citrobacter rodentium*, by inducing IL-22 production by Th17 cells that inhibits the growth of this microorganism [9]. Similarly, SFB protects in an IL-17-dependent manner against development of type 1 diabetes (T1D) in Non Obese Diabetes (NOD) mice [18], a spontaneous model of T1D. In this case, SFB-induced Th17 cells are not involved in disease pathogenesis, but appear to be protective through a mechanism that involves TGFβ [19]. These data indicate that a given gut commensal bacteria is not always beneficial or detrimental, but can have differential effects depending on the context. Furthermore, the presence of *Clostridium leptum* and *coccoides* bacteria, or colonization of mice with the human commensal, *Bacteroides fragilis* (*B. fragilis*) is associated with the induction of Tregs and prevention of dextran sodium sulfate (DSS)-induced or 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis, respectively [7,20]. The specific mechanisms by which gut commensals induce Tregs are beginning to be understood. As described below, molecules expressed or produced by commensal bacteria, such as polysaccharide A and short-chain fatty acids (SCFAs), modulate immunoregulation by acting directly on Tregs or on dendritic cells (DC). In contrast, very little is known about the mechanisms underlying the microbiota-mediated induction of $CD5^+$ Bregs that protect against EAE [15], and the specific commensal bacteria and molecules that trigger their induction remain to be identified. Moreover, although SFB colonization of germ-free mice triggers production of serum amyloid A (SAA) by cells of the terminal ileum, and SAA induces lamina propria DC to produce IL-23 and induce Th17 differentiation, the specific molecules produced by SFB that trigger this response are also unknown [9].

2. Specific interactions between commensal bacterial antigens and pattern recognition receptors of the immune system

Host–microbe interactions establish immunological homeostasis in the gut, and the molecular mechanisms underlying these interactions are currently under study. Commensals interact with the immune system via recognition of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs), including toll-like receptor (TLR) and nucleotide-binding oligomerization domain-containing protein (NOD), expressed by a variety of host cells. The gut commensal bacteria are the source of peptidoglycan that prime the innate immune response through the two cytosolic PRRs, NOD1 and NOD2, expressed by intestinal epithelial cells, paneth cells, macrophages and DC. NOD plays an important role in controlling the immune response to commensal bacteria as indicated by the fact that *Nod1*- and *Nod2*-deficient mice exhibit a weakened intestinal barrier to gut microbes due to decreased production of the antimicrobial peptides, α- and β-defensins and RegIII-gamma [21,22], and are more susceptible to DSS-induced colitis [21]. Similarly, individuals with mutations in NOD2 exhibit an increased susceptibility to Crohn's disease [23,24], and NOD1 polymorphisms are also associated with inflammatory bowel disease (IBD) [25]. Interestingly, a mutation in NOD2 in Crohn's disease patients is associated with suppression of IL-10 transcription in monocytes [26]. It is, therefore, possible that the failure to control the bacterial burden in the gut in concert with a deficiency in anti-inflammatory cytokines contribute to the generation of immune responses against gut commensals, and the subsequent intestinal inflammation in IBD patients. The specific mechanisms by which commensals trigger the immune response, and subsequent disease are, however, unknown.

Bacteroides fragilis (*B. fragilis*), a human commensal, induces Tregs and prevents development of TNBS-induced colitis [7,20]. The precise mechanisms underlying the induction of Tregs by *B. fragilis* have begun to be understood, and involve polysaccharides (summarized in Fig. 1a). *B. fragilis* prevents TNBS-induced colitis in mice through polysaccharide A (PSA) which induces IL10-producing Tregs [20]. PSA can enhance Treg function via TLR2 signaling directly in Tregs [27]. *B. fragilis* can also release PSA in outer membrane vesicles (OMVs), that are sensed by dendritic cells (DC) via TLR2 resulting in upregulation of growth arrest and DNA-damage-inducible protein (Gadd45a) on DC and an increase in IL-10 production. These DC can subsequently induce Tregs *in vivo* and prevent TNBS-induced colitis [28]. Oral administration of PSA mediates protection against EAE in an IL-10-dependent manner, and is associated with accumulation of CD103-expressing DC and Tregs in the LN draining the central nervous system [29]. Altogether, these studies show that PSA from *B. fragilis* can activate Tregs directly and/or endows DC with the ability to induce Tregs.

On the other hand, SFB is known to induce IgA [4,5] and Th17 cell [8,9] differentiation, however, the specific SFB molecules triggering these effects remain to be determined. SFB may either activate B and T cells directly, or affect intestinal cells such as epithelial cells or DC, and trigger their ability to induce IgA and Th17 cell differentiation. Recent analysis of the whole genome of SFB shows that SFB encodes four types of flagellin, three of which are recognized by TLR5 [30]. TLR5 expression is limited in the mouse gut to intestinal epithelial cells (IEC) and $CD11c^{hi}CD11b^{hi}$ lamina propria DC (LPDC). Interestingly, LPDC are critical for the generation of IgA and Th17 cells in the lamina propria, and produce

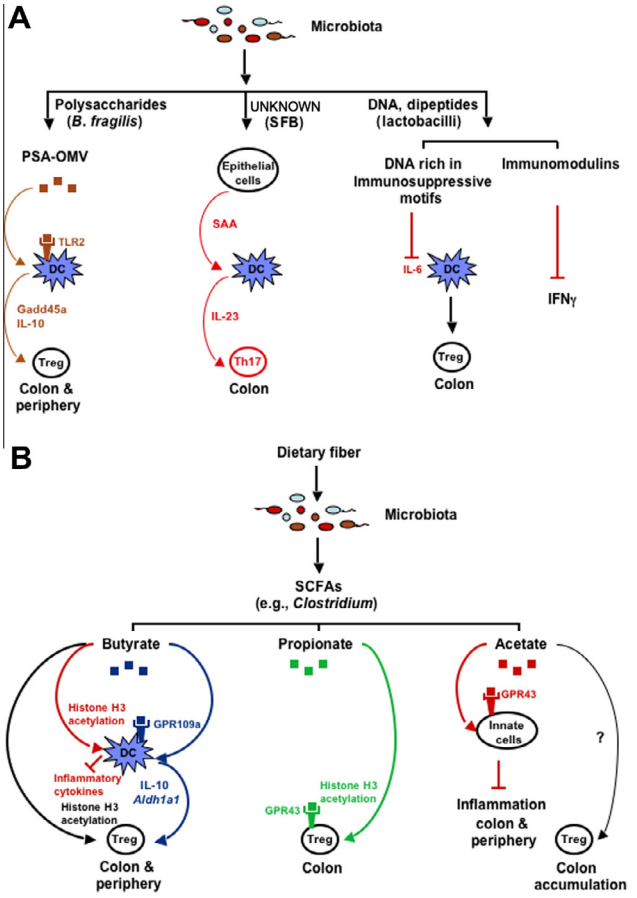

Fig. 1. Gut commensal influence on the immune response. (A) The molecules expressed and produced by commensals affect the inflammatory response and Treg differentiation. (B) Dietary fiber influences the gut microbiota composition, and in turn, the metabolites produced by commensals affect the inflammatory response and Treg differentiation/accumulation, *Aldha1*: aldehyde dehydrogenase isoform 1A1; DC: dendritic cells; Gadd45a: growth arrest and DNA damage inducible alpha; GPR: G-protein-coupled receptor; NLR4: NLR family CARD domain containing 4; PSA-OMV: polysaccharide A-associated outer membrane vesicles; SAA: serum amyloid A; SCFAs: short-chain fatty acids; TLR: Toll-like receptor; Treg: regulatory T cells.

IL-6 in response to flagellin [31]. Although some flagellated commensal bacteria may be able to evade TLR5 detection, it is possible that one or more of the flagellins produced by SFB can interact with TLR5 on LPDC or the cytosolic receptor NLRC4, and trigger the release of cytokines favoring Th17 cell differentiation. Since immune responses to flagellin(s) seem to be involved in colitis development [32], it would be interesting to determine whether SFB manipulated not to produce flagellins can still induce IgA and Th17 differentiation. In addition, the numbers of LP Th17 cells are decreased in TLR9-deficient mice indicating an adjuvant effect for commensal bacterial DNA [33], and the potential involvement of SFB DNA in this process.

3. Role of molecules produced by gut commensals in the regulation of the immune response

Commensals produce various molecules, from metabolites to immunomodulatory peptides and DNA, that could affect cells of the immune system (summarized in Fig. 1a and b). Short-chain fatty acids (SCFAs) that are metabolized by gut commensals and influence Tregs have received considerable attention recently [34–37]. Garret and colleagues were the first to show that SCFAs produced by commensals can regulate homeostasis of Tregs in the colon [34]. Oral administration (in drinking water) of the SCFAs, butyrate, acetate and propionate, in germ-free (GF) mice increases Treg numbers in the colon, but not the lymphoid organs. SCFAs appear to mediate their effect directly on colonic Tregs, since Foxp3 and IL-10 expression and suppressive function increase after culture with SCFAs *in vitro* or treatment *in vivo*. Moreover, propionate enhances Treg proliferation *in vitro*, and affects colonic Tregs via the G-protein-coupled receptor (GPR), GPR43, through inhibition of histone deacetylase (HDAC) and subsequent histone-H3 acetylation. The development of colitis in the $CD4^{+}CD45RB^{hi}$ adoptive transfer model was ameliorated by oral administration of propionate (in drinking water), correlating with higher numbers of colonic Tregs, and dependent on GPR43 expression in Tregs [34]. Interestingly, higher levels of propionate and acetate are produced by *Clostridium* cluster XIV than *B. fragilis* [34], and may mediate the ability of *C. leptum* and *coccoides* to induce Tregs [7]. Similarly, two later studies published in the same issue of *Nature* provide further evidence that SCFAs influence Treg differentiation and new insight into the molecular mechanisms underlying the effects of butyrate on Treg differentiation [35,36]. Rudensky and colleagues showed that oral administration of butyrate through drinking water in antibiotic-treated mice promoted conserved noncoding sequence 1 (CNS1)-dependent extrathymic differentiation of Tregs in the spleen and LN, but not colon [35], whereas feeding mice butyrylated starch increased Tregs in the colon. Oral administration (in drinking water) of propionate induces the new generation of splenic and colon Tregs, whereas acetate only induces accumulation of Tregs in the colon in a CNS1-independent manner (i.e., via expansion of thymus-derived Tregs, not *de novo* generation of Tregs), but not the spleen. Butyrate appears to mediate its effects, at least in part, directly on T cells via acetylation of histone H3 at the Foxp3 promoter and CNS1 enhancer. Furthermore, butyrate, and to a lesser extent, propionate, endows dendritic cells (DC) with an increased ability to induce Treg differentiation though enhanced histone H3 acetylation in DC [35]. Ohno and colleagues showed that butyrate preferentially induces colonic Treg differentiation, even under Th1- and Th17-polarizing conditions, via enhanced histone H3 acetylation at both the promoter and the conserved noncoding sequences of the Foxp3 gene locus. They also showed that feeding a butyrylated diet ameliorates colitis in the $CD4^{+}CD45RB^{hi}$ adoptive transfer model through the induction of colonic Tregs, i.e., Treg depletion abrogates protection [36]. Another study by Ganapathy and colleagues addressed the possibility that butyrate affects Treg differentiation in the colon by activation of an GPR [37], since, as discussed above, both propionate and acetate mediate their effects on colonic Tregs and the inflammatory response in DSS-induced colitis, respectively, via GPR43 [34,38]. This group showed that GPR109a signaling enables colonic macrophages and DC to induce differentiation of Tregs and IL-10-producing T cells. Moreover, splenic DC treated with butyrate induce Treg differentiation in a GPR109a-dependent manner via production of IL-10 and *Aldh1a1* [37]. This is in contrast to the data from the Rudensky group who did not find a decrease in Treg induction when using DC from GPR109a deficient mice [35]. The reason(s) for the discrepancy between the two studies is not currently clear, but underscores the complexity of the mechanisms of action of SCFAs. Butyrate has also been shown to activate GPR41 expressed by immune cells, including peripheral blood mononuclear cells (PBMC), neutrophils, monocytes and DC [39], suggesting that butyrate may also act through GPR41 expressed by colonic DC, and induce Treg differentiation in the colon through this mechanism. In summary, these studies show that SCFAs can differentially influence immunoregulation at three different levels by: (1) acting directly on pre-existing colonic Tregs via histone acetylation through GPR activation; (2) inducing naïve T cells to differentiate into Tregs via epigenetic upregulation of the Foxp3 gene through histone acetylation; and

(3) directly influencing the ability of DC to mediate Treg differentiation via histone acetylation or GPR activation. Taken together, these studies suggest that the types and levels of metabolites produced by commensal bacteria may directly affect the balance between pro- and anti-inflammatory immune cells.

The precise molecules that mediate the ability of other gut commensal bacteria, e.g., lactobacilli, to prevent autoimmune/inflammatory diseases, such as colitis and type 1 diabetes [reviewed in [40]], remain to be identified. Interestingly, although lactobacilli do not produce butyrate [41], a recent metabolomic study showed that *Lactobacilli plantarum* produces small immunomodulatory molecules, the immunomodulins (e.g., pyro-dipeptides), that exhibit anti-inflammatory activity. For example, administration of pyro-phenylalanine and pyro-tryptophan into mice suppresses production of IFNγ in spleen stimulated with LPS [42]. Furthermore, a recent study showed that the ability of commensal DNA to stimulate immune responses appears to be species-specific and inversely correlates with the frequency of immunosuppressive motif-containing DNA [43]. Genomic DNA from *Lactobacillus paracasei* failed to induce proinflammatory cytokines, such as IL-6, by lamina propria DC, rather, it induces Treg conversion *in vitro*, whereas DNA from *E. coli* or CpG oligodeoxynucleotide induces proinflammatory cytokines. This difference is apparently due to the increased frequency in the suppressive sequences, TTAGGG and TCAAGCTTGA, in the DNA of all *Lactobacillus* species tested, but not in *E. coli*.

As mentioned above, other gut commensals, such as segmented filamentous bacteria (SFB), have been implicated in the development of inflammatory/autoimmune diseases mediated by Th17 cells, including colitis, arthritis and EAE [10–12]. Several studies have shown that SFB-induced differentiation of Th17 is likely to involve mechanisms that are independent of TLR-, NOD- or ATP-signaling [9,44,45], but involves secretion of serum amyloid A (SAA) possibly by gut epithelium, and subsequent production of IL-23 by lamina propria DC [9]. However, the SFB molecules involved in induction of Th17 cells remain to be identified. Specific metabolites produced by SFB, such as SCFAs, may preferentially induce SAA and mediate Th17 differentiation. Butyrate has indeed been shown to induce SAA2 genes in intestinal epithelial cells [46]. Finally, DNA from SFB could have a paucity of immunosuppressive motif-containing DNA, and may consequently, preferentially promote intestinal immune effector cells rather than sustaining Tregs.

It would be very informative in the future to more specifically characterize the impact of various known gut commensals and their components, i.e., surface molecules, DNA composition and metabolites, on the cytokine profile of various immune cells such as Th1, Th2, Th17, B cells, Bregs, Tregs, DC and macrophages, in order to define a pattern that could predict whether a particular commensal, DNA and/or their particular metabolite ratios will induce pro- or anti-inflammatory responses.

4. Influence of the immune response on the gut microbiota

Several studies examining whether and how the immune response affects the gut microbiota composition using mice with selective immune deficiencies have found that both innate and adaptive immune cells appear to shape the composition of the gut microbiota. The composition of the gut microbiota in CD45-, Rag- and CD45Rag-deficient mice differs from wild-type (WT) mice. Furthermore, co-housing CD45-deficient and WT mice results in alteration of the microbiota composition in WT mice [47]. The data indicate that cells of the immune system influence the gut microbiota composition. Some studies have shown that cells of innate immunity influence the gut microbiota. Loss of Tbet in Rag2 deficient (TRUC) mice leads to the overproduction of TNFα by colonic DC in response to gut commensal bacteria, and generates a colitogenic microbial community [48]. TRUC mice exhibit an increased Bacteroidetes to Firmicutes ratio, and a lower proportion of Proteobacteria compared to Rag2 deficient mice. Moreover, *Klebsiella pneumoniae* and *Proteus mirabilis* recovered from fecal samples of TRUC mice were found to elicit colitis in Rag2 deficient mice [49]. A recent study has implicated group 3 innate lymphoid cells (ILCs) and the aryl hydrocarbon receptor (Ahr) in promoting gut immunity and controlling the gut commensals. Aryl hydrocarbon receptor is expressed on ILCs and regulates their maintenance and function, including IL-22 production [50]. Interestingly, Ahr deficient mice exhibit reduced levels of ILCs and IL-22, leading to the uncontrolled expansion of SFB and a subsequent increase in Th17 cells and colitis development [51]. This study suggests that ILCs control SFB levels in the gut via production of IL-22. Nucleotide-binding oligomerization domain-containing protein 2 (NOD2), an intracellular protein sensor for bacterial cell wall components, also plays an important role in the temporal development and composition of the gut microbiota. Indeed, several reports have shown that *Nod2*-deficient mice display an increased bacterial load in their feces and terminal ileum that exhibit an altered composition characterized by decreased diversity and richness. A decrease in fecal Proteobacteria [52] combined with an increase in both fecal and ileal Bacteroidetes and Firmicutes was observed in *Nod2*-deficient mice compared to the WT mice [52–54]. Interestingly, the Bacteroidetes and Firmicutes load is also higher in biopsies taken from patients with Crohn disease that are homozygous for SNP13, a NOD2 variant [54]. Moreover, the gut microbiota of *Nod2*-deficient mice predisposes these mice to the development of DSS-induced colitis. The disease risk was transmissible to WT mice conferred by cohousing WT with *Nod2*-deficient mice, while mitigated by transplanting the cecal homogenate from WT mice into germ-free *Nod2*-deficient mice [55]. These findings suggest that NOD2 plays a role in shaping the gut microbiota into a protective bacterial community, although one recent study using F2 littermates could not find any differences (22). Furthermore, colonic epithelial cells from mice deficient in the inflammasome component, NLRP6, produced lower IL-18 levels, resulting in increased levels of TM7 phyla and Bacteroidetes (*Prevotellaceae*, in particular) compared to WT mice. NLRP6 deficient mice also developed colitis. Both the unique microbiota as well as the colitis could be transferred by co-housing WT mice with NLRP6 deficient mice [56]. Finally, two studies found different effects of TLR5 on the gut microbiota. One study showed that TLR5 deficiency alters the gut microbiota by comparing TLR5 deficient and WT mice [57], whereas another study found no differences in the composition of the gut microbiota in mice deficient for MyD88, or TLR2, 4, 5, or 9 compared to WT littermates [58]. The conflicting conclusions of these two studies may be due to the fact that littermates were not compared in the first study. Microbiota drift has been observed during long-term breeding of isolated mouse colonies. Therefore, changes in microbiota composition between non-littermate WT and deficient mice could be attributed to maternal transmission rather than to a deficient immune response [58]. The use of littermates should be favored when comparing the gut microbiota between different groups of mice. Nevertheless, taken together, the data described above strongly suggest that the innate immune response has an impact on the composition of the gut microbiota.

Several studies have shown that adaptive immunity also has an influence on the gut microbiota. Using 16S rDNA deep sequencing, one recent study showed that the composition of the gut microbiota in Rag1-deficient mice is significantly different than that of their WT littermates, largely driven by an increase in Firmicutes. In contrast, MyD88$^{-/-}$TRIF$^{-/-}$ mice exhibit no differences in gut microbiota composition compared to WT littermates [59]. This study indicates that T and B cells are capable of modulating the

composition of the gut microbiota. Moreover, mice deficient for activation-induced cytidine deaminase (AID) lack IgA and appear to support abnormally high levels of SFB compared to $AID^{+/+}$ littermates. The lack of IgA in $AID^{-/-}$ deficient mice is associated with an expansion of SFB and subsequent B cell hyperplasia. Parabiosis between $AID^{-/-}$ and GFP Tg mice at 2 months of age, which allows exchange of gut-primed IgA-producing B cells from GFP Tg mice to $AID^{-/-}$ mice, reestablishes bacterial homeostasis in the small intestine by preventing the expansion of SFB [60]. In another study, the levels of SFB in SFB-monoassociated *scid/scid* mice remain high through 70 days of age whereas they were detected at very low levels in scid/+ pups. This difference appears to be associated with the lack of both acquired maternal secretory IgA in the milk from *scid* mothers and self-produced intestinal IgA, and, therefore, a failure to eliminate SFB via an IgA-mediated mechanism [61]. These data indicate that the ability to produce IgA is critical for regulation of certain gut commensal bacteria, especially SFB.

Altogether, it appears that the cells of adaptive and innate immunity can affect the composition of the gut microbiota. The fact that the gut microbiota and the immune response can influence each other suggests that an intricate relationship exists between these two entities. Therefore, further dissection of the role that various immune cells/molecules play is very important for understanding these interactions, and will be crucial for developing prevention and/or treatment strategies for inflammatory diseases.

5. Interaction between hormones, microbiota and autoimmunity

Many autoimmune diseases occur with a much greater prevalence in females than males, as indicated by a female to male incidence ratio of 50:1 for Hashimoto's disease, 9:1 for Sjogren's syndrome, 9:1 for systemic lupus erythematosus (SLE), 9:1 for antiphospholipid syndrome, 4:1 for rheumatoid arthritis and 3:1 for multiple sclerosis [62]. Although sex hormones have been shown to have an impact on immune cells and autoimmune diseases, the microbiota is emerging as an important environmental factor in the sex-based bias in autoimmunity. This is based on two recent studies examining differences in microbiota between female and male mice in the spontaneous NOD model of type 1 diabetes (T1D) in which female, but not male, mice develop high incidence of disease. These two studies demonstrate that gut commensals influence sex hormone levels in the host, that sex hormones, in turn, influence the composition of the gut microbiota and somehow the microbiota and sex hormones, specifically androgens, together collaborate to modulate the pathogenic response, and ultimately, disease development in NOD mice [63,64].

The first study by Danska and colleagues showed that a direct interaction exists between sex hormones and microbiota that has a significant impact on the development of T1D [63]. Unlike specific pathogen free (SPF) NOD mice, germ-free (GF) female and male NOD mice develop converging disease rates with similar kinetics that is due to both an increase in disease incidence from historical rates in males and a, somewhat less pronounced, decreased rate in females. These data suggest that the sex-based bias in disease development in NOD mice is driven by commensal bacteria, and that male-specific microbiota may play a protective role. Moreover, SPF male mice exhibit higher serum testosterone levels than GF males, suggesting that commensal bacteria differentially regulate testosterone production, and provide a protective effect mediated, at least in part, via microbiota metabolism of sex hormones. Conversely, sex-specific differences in the composition of the microbiota are found only after puberty, suggesting that sex hormones may affect the composition of the gut microbiota. Moreover, orally gavage of female NOD weanlings with the cecal contents from 14 week-old males altered the composition of the microbiota, increased the testosterone levels, and reduced insulitis, levels of insulin-specific antibodies, and incidence of disease in the female recipients. Interestingly, this protection from disease is abrogated by treatment with flutamide, an anti-androgen drug, suggesting that the male microbiota confers protection via an increase in testosterone available to the female recipients. Thus, manipulation of the gut microbiota of T1D-prone NOD mice at an early age may confer protection from the disease by altering sex hormone levels. This paper provides evidence for a direct effect of the microbiota on the levels of male hormones, which in turn influence disease development/progression.

The second study by Yurkovetskiy and colleagues also showed that microbiota and hormones interact, triggering protective pathways in NOD mice [64]. In this study the authors showed, using high-throughput sequencing of 16S rRNA, that pre-pubertal female and male NOD mice possess similar compositions of microbiota, but the populations begin to diverge in male, but not female, mice as they mature. In contrast, the composition of castrated male mice remained very similar to females over time, further suggesting that male sex hormones affect the composition of the gut microbiota. Interestingly, reconstitution of GF NOD mice with the Enterobacteriacae family (found in abundance by sequencing in one experiment) or SFB protects only male, not female mice, from developing T1D, suggesting that these specific bacteria can provide protection to males. Analysis of testosterone levels in GF, SPF and monocolonized male mice showed that the specific bacteria that protect males from developing disease, i.e., Enterobacteriacae and SFB, also increased testosterone levels in these mice. Finally, analysis of the gene expression pattern in pancreatic lymph nodes revealed differential expression of 40 genes in the SPF male (i.e., mice protected from disease) compared to GF male and female, and SPF female (i.e., all three develop disease) mice. The IFNγ and IL-1β pathway signatures are upregulated in SPF male mice, and an analysis of several studies previously published by other laboratories show that male NOD mice lacking IFNγ, IFNγ receptor, or IL-1β develop disease rates compared to female NOD mice, and strongly suggest that IFNγ and IL-1β-mediated signaling may be needed for protection of male mice from disease. Additionally, the authors compared the expression pattern in pancreatic lymph nodes (LN) from SPF NOD mice of 40 differentially expressed genes using a meta analysis of data from 96 different cells/tissues, and found that this pattern is most consistent with the macrophage–monocyte lineage. Moreover, pancreatic LN (more specifically CD4 and CD8 T cells) from SPF male mice exposed to SFB produce more IFNγ and IL-1β compared to female or castrated SPF male mice that may be driven by microbiota-activated macrophages.

Taken together, these two studies show that signals from both the microbiota and androgens modulate immune cells, and together they mediate the sex-based bias in T1D seen in NOD mice [63,64]. With regard to the molecular mechanisms mediating protection in male NOD mice, IFNγ seems to be a key molecule involved, but its mechanism of action is unclear and requires further investigation. However, T1D is not a sex-biased disease in humans, whereas the prevalence of systemic lupus erythematous (lupus) is much greater in women than men (9:1). The (NZBxNZW)F1 (BWF1) mouse model of lupus exhibits the same female sex bias as that found in humans. Female, but not male, BWF1 mice develop the disease, and we have preliminary data indicating that, similar to the pattern found in NOD mice, the composition of gut microbiota in BWF1 male mice differed significantly from that of mature, but not peripuberal, female mice (data not shown). These data suggest that androgens have an impact on gut microbiota composition in lupus-susceptible mice, and as shown

in the NOD model of T1D [63,64], the male microbiota may be protective. Moreover, these data may indicate that unlike men, women that are genetically predisposed to develop lupus may possess gut microbiota that fail to protect them against disease development. In the future, it will be important to determine the specific mechanisms and/or molecules produced by gut commensals that are affected by, and affect, androgens, and are involved in protection from sex-biased diseases such as lupus. This understanding will allow the development of novel strategies to prevent and/or treat sex-biased autoimmune diseases.

6. Influence of the gut microbiota on inflammatory diseases

Studies in animal models of autoimmunity suggest that the gut microbiota composition may determine whether a genetically susceptible individual will develop inflammatory/autoimmune disease (reviewed in [17]). Segmented filamentous bacteria (SFB) is implicated in Th17-mediated diseases in animal models of arthritis, experimental autoimmune encephalomyelitis (EAE), colitis [10–12], and has been shown to exacerbate experimental asthma [65]. However, SFB can protect against type 1 diabetes (T1D) [18] and from invasion by *C. rodentium* [9]. *B. fragilis*, and *Clostridium* and *Lactobacillus* species appear to be protective in animal models of EAE, colitis, arthritis, T1D and asthma [7,20,66–69]. Although dividing gut bacteria into "beneficial" and harmful bacteria may be an oversimplification of the role of microbes in the gut, accumulating evidence suggests that alterations in the gut microbiota composition, frequently referred to as dysbiosis, are associated with the development of allergies and inflammatory/autoimmune diseases, including inflammatory bowel disease (IBD), rheumatoid arthritis (RA) and T1D, in humans and animal models of disease (Table 1).

6.1. Allergic disorders

Several studies have found that infants with food allergies and eczema exhibit alterations in the relative levels of "beneficial" and potentially harmful bacteria compared to healthy infants. For example, the levels of the lactobacilli and bifidobacteria are lower, while levels of coliforms and *Staphylococcus aureus* are higher in the fecal samples from children with allergies to egg or cow's milk [70]. Infants with atopic eczema also have decreased levels of bifidobacteria and *Enterococcus* in their feces [71], but increased *Clostridium* [72]. Along these lines, a recent study showed that early lactobacilli colonization (at one week of life) appears to correlate with a decreased risk for allergy at five years of age [73]. These studies corroborate previous reports indicating that oral treatment with lactobacilli improves allergy [74–77]. Similarly, the gut microbiota also appears to be involved in asthma development. A prospective birth cohort study comparing the fecal composition at 3 weeks of age and respiratory symptoms at 6 and 12 months of age reveals an association between antibiotic use, high levels of anaerobic bacteria and wheezing during the first year of life, while high levels of *Clostridium* species, in general, are protective [78]. However, another study shows that *Clostridium difficile* colonization in 1 month-old infants is associated with the eventual development of asthma at 6–7 years of age [79]. Taken together, these data suggest that specific species of intestinal commensal bacteria may play either a pathogenic or protective role in the development of allergies occuring in the gut or at sites distant from the gut, such as the lung and skin.

6.2. Inflammatory bowel disease

The inflammatory bowel diseases (IBD), Crohn's disease (CD) and ulcerative colitis (UC), differ from classical autoimmune disorders in that they reflect a disordered host response against the intestinal microbiota, rather than a true autoimmune reaction against a self protein [80]. IBD is thought to arise from an abnormal immune response to components of the intestinal microbiota [81]. A spectrum of alterations in the gut microbiota is well described in IBD patients, including increases in *E. coli* [82,83] and lower levels of specific types of *Bacteroides* [84,85] and Firmicutes [86–88], in particular, *Faecalibacterium prausnitzii* (*F. prausnitzii*) from the Clostridia class and Firmicutes phylum [82,88] compared to healthy controls. Although a particular etiologic agent has not been found to date, a subset of CD patients was found to harbor a potentially proinflammatory strain of adherent-invasive *Escherichia coli* (*E. coli*) in their small intestine [89]. In a recent study, *Bifidobacterium* was found to be increased in biopsies of active UC while *Lactobacillus* was increased in biopsies of active CD patients compared to healthy controls, and *F. prausnitzii* was decreased in both feces and biopsies of UC and CD patients [90]. Although treatment with antibiotics may benefit patients with IBD, two studies indicate that antibiotic treatment early in life may increase the risk of IBD [91,92]. Altogether, these data suggest that gut commensals can play either a pathogenic or protective role in IBD.

6.3. Rheumatoid arthritis

Rheumatoid arthritis (RA) patients treated with minocyline, a broad-spectrum antibiotic, exhibit significant disease improvement [93]. Moreover, alterations in the gut microbiota have been demonstrated in patients with early RA [94,95]. One study comparing fecal samples from early RA patients to patients with fibromyalgia showed that bifidobacteria and *B. fragilis* are decreased in the gut of RA patients [94], suggesting that alterations in the abundance of these two commensal species may influence the pathogenesis of the disease. Interestingly, another study identified *Prevotella copri* (*P. copri*) as a potential inducer of RA [95]. Indeed, the authors found alterations of the gut microbiota in untreated newly diagnosed RA patients compared to healthy individuals that is characterized by an enrichment in *P. copri* and a decreased abundance of *Clostridium* XIV and Bacteroidetes. These data suggest that commensal bacteria may also play either a pathogenic or protective role in RA.

6.4. Type 1 diabetes

Studies in animal models of type 1 diabetes (T1D) have identified associations between disease development and changes in the gut microbiota [96–98]. Translating this observation into a clinical context, four recent studies in humans show that the composition of the gut microbiota may be altered in individuals at high risk of developing T1D, i.e., those who express high-risk HLA alleles and have at least two different diabetes-associated autoantibodies in their serum [99–102]. The first observation came from a cohort of Finish children with high-risk HLA for T1D, where fecal samples from subjects seropositive for two autoantibodies were compared to seronegative controls (referred to as healthy) [99]. Pyrosequencing of the stool microbiota 16S rRNA revealed that Bacteroidetes sequences increase over time while Firmicutes sequences decrease in the children with autoantibodies, whereas the pattern was reversed in the healthy controls. Interestingly, *Clostridium* species and *B. fragilis* are decreased in stool samples from seropositive children. Moreover, the microbiome from seropositive children tended to have decreased bacterial diversity and reduced stability over time [99]. In a second study, the same group found that butyrate-producing bacteria are decreased in the seropositive children [100]. Two subsequent larger studies that also analyzed pyrosequencing of fecal samples from seroconverted and healthy children matched for age, sex and HLA-DQB1 genotype (high-risk for T1D)

Table 1
Changes in microbiota composition associated with allergy and inflammatory/autoimmune diseases in patients and corresponding animal models.

Microorganism(s)	Disease(s)	References
	Asthma/allergies	
SFB ↑	Asthma ↑	[65]
Lactobacilli/bifidobacteria ↓ Coliforms/*Staphylococcus aureus* ↑	Egg/milk allergies ↑	[70]
Bifidobacteria/*Enterococcus* ↓	Atopic dermatitis/eczema ↑	[71]
Clostridium ↑	Atopic dermatitis/eczema ↑	[72]
Lactobacilli ↑	Allergy ↓	[73–77]
Clostridium species ↑	Asthma ↓	[78]
Clostridium difficile ↑	Asthma ↑	[79]
Lactobacilli ↑	Allergy/asthma ↓ (mouse)	[115]
Clostridium leptum ↑	Asthma ↓ (mouse)	[116]
	Inflammatory diseases	
Escherichia coli ↑	IBD (CD and/or UC patients) ↑	[82,83,89,108]
Bacteroides ↓	IBD (UC-CD patients) ↑	[84,85]
Firmicutes ↓	IBD (UC-CD patients) ↑	[86–88]
Firmicutes prausnitizi ↓	IBD (UC-CD patients) ↑	[82,88]
Bifidobacteria ↑ *Firmicutes prausnitizi* ↓	IBD (UC patients) ↑	[90]
Lactobacillus ↑ *Firmicutes prausnitizi* ↓	IBD (CD patients) ↑	[90]
Firmicutes prausnitizi ↑	TNBS-colitis (mouse) ↓	[112]
SFB ↑	CD45RBhi-colitis (mouse) ↑	[11]
Prevotella copri ↑	DSS-colitis (mouse) ↑	[95]
	Autoimmune diseases	
Bifidobacteria/*Bacteroides fragilis* ↓	RA vs fibromyalgia patients ↑	[94]
Prevotella copri ↑ *Clostridium XIV*/ Bacteroidetes ↓	RA patients ↑	[95]
SFB ↑	RA, EAE (mouse) ↑	[10,12]
SFB ↑	T1D (NOD mouse) ↓	[18]
Bacteroidetes ↑ Firmicutes ↓	High risk T1D patients ↑	[99,101,102]
Clostridia/*Bacteroides fragilis* ↓	High risk T1D patients ↑	[99,101,102]

CD: Crohn's disease; DSS: dextran sodium sulfate; EAE: experimental autoimmune encephalomyelitis; IBD: inflammatory bowel disease; NOD: non-obsese diabetes; RA: rheumatoid arthritis; SFB: segmented filamentous bacteria; T1D: type 1 diabetes; TNBS: trinitrobenzene sulfonic acid; UC: ulcerative colitis.

confirmed the previous findings [101,102]. In conclusion, higher levels of bacteria from the Bacteroidetes phylum and the *Bacteroides* genus combined with lower levels of butyrate-producing bacteria, including members of *Clostridium* clusters IV and XIVa that are associated with Treg induction in mice [7] and anti-inflammatory properties, are found in seroconverted children.

6.5. Systemic lupus erythematosus

The relationship between systemic lupus erythematosus (SLE) and the gut microbiota is still not well-characterized. One study showed that germ-free lupus-prone NZB mice fed an antigen-free diet exhibit less renal disease, while another study finds that germ-free NZB mice have lower IgG levels, but higher anti-nuclear antibodies (ANA) [103,104]. We have found that feeding of *Lactobacillus reuteri* to B6Sle.123 and (NZBxNZW)F1 mice (two spontaneous mouse models of SLE) prevented lupus development, increased survival, and increased the levels of peripheral Tregs (data not shown). Since levels of Tregs are impaired in both B6Sle1 and (NZBxNZW)F1 mice [105,106], there is a possibility that administration of lactobacilli provided protection by modifying the gut microbiota composition towards one that favors Treg induction. The immunosuppressive motif-containing DNA expressed by lactobacilli could also affect Treg induction directly as shown previously [43]. Finally, immunomodulins produced by lactobacilli could potentially influence Tregs and enhance their ability to control effector cell responses [42]. To date, no analysis of the gut microbiota of SLE patients has been performed. It would be particularly interesting to compare the microbiota composition in lupus patients that are in disease remission versus patients with active disease to determine whether lupus flares are associated with variations in particular gut commensals.

6.6. What does it all mean?

The studies described above indicate that alterations in the gut microbiota are associated with development of many allergic disorders and inflammatory/autoimmune diseases. The mechanisms underlying the impact of the microbiota on disease outcome are not yet understood, but appear to involve increases or decreases in various gut commensals that may be more conducive to pathogenic or protective responses, most likely in the context of genetic susceptibility. Although SFB is associated with induction of a number of diseases in mice, its relevance to human disease is in question. A recent study did not detect evidence of the SFB genome in the MetaHIT database (largest database for metagenomics of the human intestinal tract) or the Human Microbiome Project metagenomic database, suggesting that SFB is either not present or is below the detection level in humans [107]. Identifying an analogous gut commensal in humans that may trigger a pathogenic immune response responsible for disease development may provide the basis for novel therapies. However, many gut commensals appear to be associated with an increase in inflammatory/autoimmune and allergic diseases (Table 1). A *Prevotella* species such as *P. Copri* is elevated in untreated newly diagnosed RA patients, suggesting that this commensal could potentially induce disease. This is corroborated by experiments in mice showing that oral gavage of antibiotic-treated mice with *P. copri* exacerbates DSS-induced colitis [95], suggesting that a *Prevotella*-enriched microbiome has the tendency to support inflammation. Adherent-invasive *E. coli* (AIEC) are present in the intestinal mucosa of CD and UC patients, but not healthy patients [83,89,108]. AIEC isolated from IBD patients are able to invade epithelial cells [109] and survive in macrophages within autophagosomes [108–110]. Moreover, AIEC can induce macrophages to produce high levels of IL-6, TNF-α, and IL-1β via NLRP3-inflammasome [108]. Since CD patients exhibit lower expression of NLRP3 [111], there is a possibility that a weak innate inflammatory response is generated early in the disease process in IBD patients, resulting in impaired clearance of AIEC and chronic inflammation.

Decreases in *Clostridium* species, *B. fragilis* and butyrate-producing bacteria appear to be common to many inflammatory/autoimmune diseases (Table 1), i.e., T1D, IBD, and RA. The decrease in *F. prausnitzii* found in IBD patients is of interest because this bacteria is butyrate-producing, and its oral administration reduces the severity of TNBS-induced colitis in mice [112]. It is possible that decreases in these types of gut microbiota results in the impairment of Tregs, since the types of commensals, or their metabolites, that are decreased [34–36] have been associated with Treg induction in animal models [7,27]. Therefore, the defect in immunoregulation observed in T1D patients [113,114] may be tied to alterations in the composition of gut microbiota, since certain bacteria [7,20] and their metabolites associated with Treg induction [34–36] are decreased in individuals that seroconvert (T1D).

C. leptum, lactobacilli and bifidobacteria appear to be "beneficial" for allergy, since there is an inverse relationship between allergy manifestation and the levels of these gut commensals (Table 1). Although the mechanisms behind the protection are unknown, a recent study reported that oral exposure of adult mice to house dust induces *Lactobacillus* enrichment of their gut microbiota, and leads to a decreased Th2 response against allergens in

their airways [115]. *C. leptum* may also mediate its protective effect via induction and/or activation of Tregs, since oral feeding of *C. leptum* in mice increases Treg numbers in spleen and mediastinal LN, and attenuates allergic airway inflammation [116]. Taken together, these studies indicate that the gut microbiota can affect sites, in this case the respiratory system, distant from the gut. One postulated mechanism by which gut commensals mediate their effect at distant sites is through production of SCFAs that can be found in the circulation of the host upon fermentation of dietary fiber [117]. Mice fed a high-fiber diet have increased circulating levels of SCFAs and are protected against allergic airway disease. Furthermore, treatment of mice with propionate enhances bone marrow hematopoiesis of DC precursors that eventually seed the lung, but are unable to activate Th2 effector cells, thereby preventing the development of allergic inflammation of the airways [117]. Since treatment of mice with butyrate influences systemic Treg differentiation [35], SCFAs may mediate their immune modulating effects at distant sites via induction of Tregs. And since many species of gut commensals produce SCFAs with the same structure, the only differences among commensals is likely to be the quantity of each type that is produced.

In patients with established allergic and inflammatory/autoimmune disease, it is difficult to determine whether changes in microbiota composition are a consequence or a cause of the disease. Disease concordance in many autoimmune diseases is 30–50% in monozygotic twins indicating that both genetic and environmental factors govern development of disease, and therefore, alterations in gut microbiota could play an important role. In individuals at high-risk of developing T1D, changes in microbiota can be detected before disease onset (i.e., elevated glucose), but concomitant with the production of autoantibodies, suggesting that components of the microbiota may have an impact on the immune response and progression to full-blown disease. On the other hand, the inflammatory response that leads to the production of autoantibodies may also affect the gut microbiota. Interventional studies in humans via manipulation of the gut microbiota through the use of probiotics [118,119] or fecal transplants [120,121], have had a positive impact on disease status in IBD patients, and treatment with probiotics improves allergic disorders [74–77]. These findings, at least, suggest that the gut microbiota can be altered to have a positive impact on these types of disease.

Finally, the fungal and viral components of the microbiome should not be ignored, since these microorganisms have also been shown to interact with the immune system and influence heath and disease in mouse models of colitis [122,123]. A relationship between the immune response and the human virome has been identified, as indicated by increases in the total viral load of the human virome during immunosuppression of transplant patients, but not in the bacterial microbiome [124]. The molecules responsible, as well as the precise mechanisms of action, mediating changes in the fungal and viral components of the microbiome, and subsequent effects on the immune response and disease development remain to be assessed. This knowledge will not only help us understand their contribution to disease susceptibility, but also aid us in the development of novel therapies.

7. Using gut microbiota or its components in therapies for the treatment of autoimmunity and allergy

Human gut microbiota, i.e., specific commensal bacteria or total fecal transplant, is a new class of therapeutics under consideration for the prevention and/or treatment of inflammatory diseases. Fecal transplant is already used as a salvage treatment for *C. difficile*, and has been shown to be the most effective treatment for recurrent infection [121]. Two groups have developed a synthetic stool mixture [125] and a capsule containing donor bacteria, i.e., a fecal pill [126], that are currently under study for treating this clinical condition. A systematic review of the literature concerning the use of fecal transplantation for the treatment of IBD was performed in 2012. While 17 articles were found, nine were small case studies of patients receiving fecal transplants for the management of IBD or for the treatment of infectious diarrhea in IBD, and none were conducted as controlled clinical trials [120]. The majority of patients (15/24) treated for their IBD underwent disease remission without side effects [120], suggesting that fecal transplants may represent a safe alternative treatment for IBD. Since then, two pilot studies of fecal transplant in children with IBD have been conducted, with one showing remission in 7 out of 10 patients with Crohn's disease [127], while the second demonstrated that 3 out of 9 children with ulcerative colitis (UC) experienced remission one week following the administration of fecal enemas [128]. Finally, a third study evaluated the impact of fecal transplant on IBD in 5 adult patients with active UC refractory to standard therapy and the corresponding temporal influence on gut microbial communities. Only one patient exhibited an improved clinical score, but he did not achieve remission. At 12 weeks post-transplantation, his gut microbiota was similar to the donor, including donor-derived SFCA-producing *F. prausnitzii*, *Rosebura faceis* and *Bacteroides ovatus* [129]. In contrast, the microbiota of the other patients were very different, including underrepresented phyla (Bacteroidetes, Firmicutes and Verrucomicrobia) and an abundance of Enterobacteriaceae and Lacnospiraceae [129]. Contrasting results between pediatric and adult studies suggest that early intervention may be more beneficial. Furthermore, the identification of predictive markers for disease development may provide a window of opportunity to intervene with preventative strategies to prevent the development of active disease. In the case of individuals at risk for developing T1D, high-risk HLA expression and seropositivity can be used as predictive markers. Preventative fecal transplants from healthy relatives to high-risk individuals exhibiting signs of microbiota alterations could potentially protect the latter from developing T1D. One could envision that similar procedures could be performed for other autoimmune diseases associated with altered microbiota, such as rheumatoid arthritis [94], SLE and multiple sclerosis. After excluding the presence of gut associated pathogens, the use of gut commensal bacteria has been considered to be generally safe. However, there have been some safety concerns, including the development of transient systemic toxicity [129] or even sepsis using live bacteria in the case of immunocompromised and/or severely ill patients. Patients who contract *C. difficile* under these acute conditions are the most likely to need salvage therapy, often failing multiple antibiotic therapies. We have safely administered transplanted stool from a prescreened family member under these clinical conditions, providing life saving results. In our experience, no short-term sequellae have been encountered to date (data not reported). However, in long-term clinical disorders, such as UC, CD or other inflammatory/autoimmune disorders the direct use of prebiotics or metabolites from commensal organisms may be safer and more effective. Indeed, oral administration of acetate ameliorates DSS-induced colitis in mice [38] and *Lactobacillus rhamnosus* GG supernatant has been shown to improve intestinal barrier function, protecting against the development of alcoholic liver disease [130,131]. Finally, several trials have used prebiotics, food ingredients that stimulate the growth/activity of commensal bacteria, to treat or maintain remission of IBD. Those prebiotics included fiber from Plantago Ovata (PO) seeds [132], germinated barley food-stuff (GBF, a by-product of the brewing industry) [133,134], and oat bran, i.e., all forms of dietary fiber which ferment into SCFA. These therapies have demonstrated promise as IBD treatments through their ability to maintain remission of UC. Interestingly, treated patients in one trial demonstrated elevated SCFA stool levels [135]. Future studies

testing the impact of various metabolites on different autoimmune and allergic diseases should be conducted to determine the effective bacterial components, avoiding the negative impact of treating with live bacteria. However, we should keep in mind that commensal metabolites that are effective for IBD may not be effective for other diseases. From a clinical standpoint, efforts to modulate the enteric and other mucosal flora offer an attractive alternative to pharmacologic interventions focused on altering the immune system. Current therapies taking broad swipes at the intact immune system have unintended consequences, such as opportunistic infections, activation of autoimmune events, or even induction of malignancies. Finally, the identification of susceptibility genes and other biomarkers for early disease detection offer the ability to target individuals in a pre-clinical phase of disease. If microbiota manipulation through prebiotics, probiotics, or fecal transplant is confirmed to be safe, this treatment modality may offer a preventative solution to hold off or reduce the severity of a host of inflammatory/autoimmune disorders.

8. Concluding remarks

A number of studies suggest that alteration in the gut microbiota may be responsible for the development of multiple inflammatory diseases through the creation of an imbalance between pro-inflammatory and anti-inflammatory/regulatory immune cells. Although the specific molecules produced by commensal bacteria that trigger inflammatory responses in the gut are still not well-characterized, a number of microbiota-associated molecules, including surface molecules such as PSA from *B. fragilis* and metabolites such as SCFAs produced by a wide variety of gut commensals including Clostridia, are now known to regulate inflammatory responses through the induction of Tregs. Moreover, immunomodulins produced by and immunosuppressive DNA sequences present in lactobacilli have been shown to mediate immunoregulation. Accumulating evidence suggests that SFB is a critical component of the microbiota that drives disease development in a number of Th17-mediated mouse models of inflammatory/autoimmune diseases, and determining whether the human microbiota contains a homologous microbe with similar function should be a major focus of future work. Moreover, it will be critical to determine how SFB, or the potential human homologous commensal(s), induce Th17 cells, including the cells and molecules involved, in order to develop novel strategies for the prevention and/or treatment of Th17-mediated diseases. Androgens have also recently been identified as part of the picture, since they can both affect the gut microbiota and be affected by them. Future work aimed at characterizing, in detail, the impact of commensal by-products on the immune and hormonal systems, not only in the gut but at distant mucosal sites, will be crucial for developing therapies for diseases associated with altered gut microbiota. Furthermore, understanding the environmental factors and the genetic influences that shape the composition of the gut microbiota will be important for the development of strategies geared toward manipulating the microbiota of individuals at risk for developing disease.

Although the gut microbiota appears to play an important role in controlling both gut specific as well as distant immune responses [10,117,136], understanding the role that local microbiota plays on local immunity and subsequent disease that can result from alterations in microbiota at the site is vitally important. Naik et al. have demonstrated that resident commensal bacteria in the skin controls the local immune response in a manner similar to that previously described in the gut [137]. This concept is likely to apply to all mucosal sites, and implies that alteration in the local microbiota may play a role in various diseases. Analysis of the microbiome at sites other than the gut in healthy versus sick individuals will, therefore, be critical in order to gather information about the interaction of resident commensals and the immune response at local sites. This will aid in the design of novel therapies aimed at reestablishing a protective local microbiota, and treating pathogenesis resulting from an alteration in the microbiota. Customized therapies directed at recolonization of specific sites with particular beneficial commensals or their by-products certainly seems to be the wave of the future.

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